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(54) PROCESS FOR PRODUCING TRANSFORMED CELL

(57) A process for producing transformed cells by introducing foreign genes into target cells through piercing, which comprises the step of culturing the target cells having the foreign genes injected thereinto in the presence of a cell adhesion-active substance; and a kit for producing transformed cells suitable for use in the above method and containing as the essential ingredients the cells to be transformed with foreign genes by this method and a cell adhesion-active substance.

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Description

TECHNICAL FIELD

5 The present invention relates to a method for production of transfected cells, more particularly, a method which makes possible to effectively transfer a foreign gene into target cells in the field such as cell technology, genetic engineering, developmental engineering and the like.

BACKGROUND ART

10 As a method for transferring a foreign gene into target cells, there are known a calcium phosphate method, a DEAE-dextran method, a liposome method, an electroporation method, a microinjection method, a particle gun method and the like. All of these methods have advantages and disadvantages in respect of manipulation procedures, efficacy, damage on cells and the like. Among these methods, a perforation method such as an electroporation method, a micro-
 15 injection method, a particle gun method and the like can easily handle cells without using special reagents and have good transfer efficacy. However, damage of cells by perforation can not be avoided.

The object of the present invention is to provide a method for improving the transfer efficacy when a foreign gene is transferred into target cells by a perforation method to produce transfected cells.

20 SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for production of transfected cells and is characterized in that said aspect includes a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance, in a method for production of a transfected cell using
 25 a perforation method.

The second aspect of the present invention relates to gene-transferred cells which are produced by the method of the present invention.

The third aspect of the present invention relates to a kit for production of transfected cells, which is used for a method for production of transfected cells according to the first aspect of the present invention and is characterized in
 30 that said aspect contains a cell-adhering active substance.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is characterized in that, after a foreign gene is transferred into target cells
 35 using a perforation method, the cell is cultured in the presence of a substance having the cell adhesive activity.

As used herein, the perforation method means a method for injection of a gene by perforating a cell wall, including an electroporation method, a microinjection method, a particle gun method and the like. The electroporation method is as described in, for example, Tanpakushitsu, Kakusan, Koso, volume 31, page 1591-1603 (1986). The microinjection method is as described in, for example, Cell, volume 22, page 479-488 (1980). The particle gun method is as described
 40 in, for example, Technique, volume 3, page 3-16 (1991). These methods include the known methods used for transferring a gene into cells.

For cells used in these perforation methods, for example, animal cells may be prepared according to a known method ["Shin-Seikagaku Jikkenkoza 18, Saibobaiyogijyutsu", 1st edition (1990), edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin] or cultured animal cells may be used.

45 As used herein, a cell-adhering active substance refers to a substance having the cell-adhering activity, that is, the activity to make target cells adhere to a cell, or to an extracellular matrix which is a substance filling a space between cells in the tissue, or to a material such as plastic, glass and the like. In the present invention, any substances having the activity can be used as long as they give no adverse effects on transfection of target cells. Such the activity is to fix cells, for example, to a culture wear covered with a cell-adhering active substance while maintaining the cell in its form,
 50 or in the spreaded form, that is, in the changed form after the cell has been spreaded in one or more directions.

Attachment between the cell-adhering active substance and the target cell can be assayed using a conventional method. The method includes, for example, a method described in Nature, 352: 438-441 (1991). Briefly, the cell-adhering active substance covers a plastic dish and a population of cells to be assayed is put into medium, allowing to stand for 30 minutes to 2 hours. After this incubation period, non-adhered cells are recovered, counted and assayed for viability. Cells adhered to the cell-adhering active substance are recovered using trypsin or a cell dissociation buffer (for
 55 example, Gibco), counted and tested for viability. Then, a proportion of adhered cells is calculated and compared with standard or standard control such as a plastic dish covered with bovine serum albumin (BSA). A combination of cell-adhering active substance/cell can be determined by substantial adhesion of the target cell with the cell-adhering active substance assayed. In addition, the cell-spreading activity can be determined by observing under a microscope a

change in the form before adhered cells are dissociated using trypsin or a cell dissociation buffer, in the above procedures.

Examples of the cell-adhering active substance include, for example, a cell-adhering active polypeptide or a functional equivalent thereof and a cell-adhesive synthetic polymer.

5 Examples of the polypeptide, used in the present invention, having the cell-adhering activity include a cell-adhering active polypeptide such as invasins, polylysine and the like other than that derived from extracellular matrix, for example, a polypeptide showing the cell-spreading activity described in JP-A 2-311498, for example, components of an extracellular matrix such as fibronectin, laminin, collagen, vitronectin, osteopontin, thrombospondin, tenascin and the like. The extracellular matrix components can be prepared from a natural or cultured source by the known method [International
10 Journal of Cancer, volume 20, page 1-5 (1977); Journal of Biological Chemistry, volume 254, page 9933-9937, (1979); "Zoku-Seikagaku Jikkenkoza, volume 6, Saibokokkaku no Kozo to Kino (Structure and Function of Cell Skeleton) (last volume), (1st edition) (1986) edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin; Cell Structure and Function, volume 13, page 281-292 (1988); Journal of Biological Chemistry, volume 264, page 18202-18208 (1989); and Journal of Biological Chemistry, volume 260, page 12240-12245 (1985)]. The cell-adhering active polypeptide may
15 be substantially purified extracellular matrices exhibiting the cell-adhering activity, substantially purified extracellular matrix fragments or a mixture thereof. More particularly, proteins and polypeptides having the cell-adhering activity or the cell-spreading activity, or a functional equivalent thereof may be used.

As these cell-adhering active polypeptides, substantially purified natural polypeptides, polypeptides from enzymological or chemical degradation of the natural polypeptides, or the similar polypeptides made by genetic engineering
20 may be used. Further, materials obtained by altering these polypeptides without impairing the function, that is, the cell-adhering activity or the cell-spreading activity may be used. In the present invention, even when the amino acid sequence of a polypeptide from natural origin has deletion, substitution, addition and/or insertion of an amino acid, as long as the polypeptide has the desired cell-adhering activity or the cell-spreading activity, it is referred to as a functional equivalent of a polypeptide having the natural amino acid sequence. That is, it is known that naturally occurring proteins
25 include proteins of which amino acid sequences have mutation such as deletion, insertion, addition, substitution and the like of an amino acid due to modification reaction in the living body after production or during purification, in addition to proteins having a change in the amino acid sequence due to polymorphism or mutation of genes encoding those naturally occurring proteins and that, regardless of these, there are proteins exhibiting the physiological and biological activity substantially equivalent to that of proteins having no mutation. Like this, even when there is a structural difference
30 between polypeptides, as long as they share the common main functions, they are called polypeptides having the functionally equivalent activity.

This is also true where the above mutations are artificially introduced into the amino acid sequence of proteins. In this case, more variety of mutants may be made. As long as these mutants exhibit the physiological activity substantially equivalent to that of proteins having no mutation, they are interpreted to be a polypeptide having the functionally equivalent
35 activity.

For example, in many cases, a methionine residue present at a N-terminal of a protein expressed in *Escherichia coli* is said to be removed by an action of methionine aminopeptidase, thus, generating both proteins having a methionine residue or those having no methionine residue depending upon the kind of proteins. However, whether or not a protein has a methionine residue does not affect on the protein activity in many cases. In addition, it is known that a
40 polypeptide where a certain cysteine residue is substituted with a serine residue in the amino acid sequence of human interleukin-2 (IL-2) retains the interleukin-2 activity [Science, volume 224, page 1431 (1984)].

Further, upon production of proteins by genetic engineering, it is frequently conducted that the proteins are expressed as a fused protein. For example, in order to increase an amount of an expressed protein of interest, it is conducted that the protein is expressed by adding a N-terminal peptide chain derived from other protein to a N-terminal
45 of the protein of interest, or adding a suitable peptide chain to a N-terminal or a C-terminal of the protein of interest to facilitate purification of the protein of interest by using a carrier having the affinity to the added peptide chain.

In this respect, the related biotechnological techniques have progressed and, as the result, deletion, substitution, addition or other modification of an amino acid in a functional area of a subject can be routinely carried out. Then, the resulting amino acid sequence may be routinely screened for the desired cell-adhering activity or the cell-spreading
50 activity according to the above method.

Polypeptides having the cell-adhering activity may be an artificial polypeptide containing, in the molecule, the amino acid sequence necessary for the cell-adhering activity, for example, the amino acid sequence may be selected from the amino acid sequence represented by SEQ ID: No. 1 (RGDS), the amino acid sequence represented by SEQ ID: No. 2 (CS1) and the amino acid sequence represented by SEQ ID: No. 6 (central sequence of laminin, YIGSR).
55 These polypeptides can be prepared in a large amount by a genetic engineering method or chemical synthesis method and may be used as a purified polypeptide.

Examples of the artificial polypeptide having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 include a polypeptide represented by SEQ ID: NO. 7 described in JP-A 1-180900. The polypeptide can be prepared using *Escherichia coli* HB101/pTF1409 (FERM BP-1939) according to a method described in JP-A 1-180900. In

addition polypeptides represented by respective sequence ID numbers in the sequence list shown in Table 1 below can be prepared according to a genetic engineering method described in each specification.

In addition, a plasmid HB101/pCHV90 contained in *Escherichia coli* HB101/pCHV90 in Table 1 can be prepared using *Escherichia coli* HB101/pHD101 (FERM BP-2264) and *Escherichia coli* JM109/pTF7021 (FERM BP-1941) according to a method described in JP-A 5-271291.

Table 1

Laid Open publication	SEQ ID: No.	Living bacterium (<i>Escherichia coli</i>)	Accession No.
JP-A 1-206998	8	JM109/pTF7021	FERM BP-1941
JP-A 1-261398	9	HB101/pTF1801	FERM P-9948
JP-A 2-97397	3	JM109/pTF7221	FERM BP-1915
JP-A 2-152990	10	JM109/pTFB800	FERM BP-2126
JP-A 2-311498	11	HB101/pCH101	FERM BP-2799
JP-A 3-59000	12	JM109/pCF406	FERM P-10837
JP-A 3-232898	13	HB101/pCE102	FERM P-11226
JP-A 4-54199	14	JM109/pTF7520 +VN-IN.TAA	FERM P-11526
	15	JM109/pTF7520 +Col ^{X1}	FERM P-11527
JP-A 5-271291	16	HB101/pCHV179	FERM P-12183
	17	HB101/pCHV90	
	18	HB101/pCHV89	FERM P-182
JP-A 5-97698	19	JM109/pTF7520ColV	FERM BP-5277
JP-A 5-178897	20	JM109/pYMH-CF • A	FERM BP-5278

Alternatively, artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 can be chemically synthesized. For example, PolyRGDS described in JP-A 3-173828 can be synthesized and used.

Examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 include a polypeptide represented by SEQ ID: No. 4 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using *Escherichia coli* HB101/pHD102 (FERM P-10721) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 2 may be chemically synthesized according to a method described in JP-A 3-284700.

Further, examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 and the amino acid sequence represented by SEQ ID: No. 3 include a polypeptide represented by SEQ ID: No. 21 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using *Escherichia coli* HB101/pCH102 (FERM BP-2800) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 5 described in JP-A 3-284700 is a polypeptide containing, in the molecule, the amino acid sequences of SEQ ID: No. 1 and 2 and the polypeptide can be prepared by genetic engineering using *Escherichia coli* HB101/pCS25 (FERM P-11339) according to a method described in JP-A 3-284700.

As described above, examples of the polypeptides used in the present invention are cell-adhering active polypeptides containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2. As the polypeptide, a polypeptide obtained by covalently binding a polypeptide derived from a cell adhesion domain of human fibronectin ["Fibronectin", page 47-121 (1989), edited by Mosher, D.F., published by Academic Press] with a CS1 polypeptide derived from the same (ibid), a polypeptide derived from a heparin binding domain (ibid) containing a CS1 polypeptide, or a polypeptide derived from cell adhesion can be used, and they can be made by genetic engineering, respectively. For example, respective necessary regions are taken out from a vector containing a DNA encoding a cell adhesion domain-derived polypeptide, a vector containing a DNA encoding a CS1 polypeptide, and a vector containing a DNA encoding a heparin binding domain-derived peptide containing a CS1 polypeptide, respectively, and they can be used alone or in combination thereof to make a vector expressing a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

When a polypeptide where a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 are covalently bound is made, a covalent bonding between polypeptides may be a direct bonding or an indirect bonding, for example, an indirect bonding via a spacer. A spacer is an insertion sequence for adjusting an intermolecular distance in each region. As the spacer, an arbitral peptide chain can be used, for example, a sequence upstream of a CS1 region in fibronectin molecule. The spacer sequence can be easily introduced therein by genetic engineering.

The cell-adhesive synthetic polymers include the known poly-N-p-vinylbenzyl-D-lactonamide (PVLA).

In the present invention, the target cell include, but being not limited to, hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte, cancer cell and the like.

Examples of the foreign gene include, but being not limited to, nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes). In the present invention, the foreign gene may be inserted into a vector.

Examples of the vector are retrovirus vector, adenovirus vector, vacciniavirus vector, herpesvirus vector and the like.

According to the present invention, a target cell into which a foreign gene has been transferred by a perforation method according to a conventional method can be cultured in the presence of a cell-adhering active substance to effectively obtain transfected cells with a transferred gene. A cell culture method may be selected from the known methods depending upon a cell used. For example, when cell culturing is performed in the presence of a cell-adhering active polypeptide, 250 to 2000 $\mu\text{g/ml}$ of the cell-adhering active polypeptide may be used in a culture medium to culture it according to a conventional method.

Particularly, culturing is preferably carried out using a culture wear covered with a cell-adhering active substance. The culture wear refers to any wear normally used for cell culture, for example, a culture dish, a culture wear using a microcarrier, and a culture wear using fibrous hollow fibers. The culture wear may be covered with the substance by coating or spraying. For example, the culture wear may be easily covered with the cell-adhering active substance. The culture wear may be easily covered with the polypeptide by dissolving it in a suitable solution such as a phosphate buffered saline (PBS), adding the solution to the culture wear and allowing to stand for a suitable period of time. An amount of the polypeptide with which the culture wear is covered may be selected from a range of 50 to 1000 pmol/cm^2 , suitably 150 to 600 pmol/cm^2 .

Transfected cells which have been cultured in the presence of the cell-adhering active substance can be obtained from a culture according to a conventional method. Thus, transfected cells can be produced effectively.

The resulting transfected cells are useful for production of useful substances by cells using gene recombination techniques, exploitation of disease models, gene therapy and the like. Thus, transfected cells can be effectively produced according to the present invention.

In addition, the present invention can be simply carried out by using a kit containing a cell-adhering active substance. The cell-adhering active substance to be contained in the kit may be in a form of solutions or lyophilized powders. The kit may contain a buffer for dissolving or diluting the cell-adhering active substance, a cell culture medium, a cell culture wear and the like. For example, a transfected cell can be simply produced by preparing a kit combining polypeptides, PBS for diluting the polypeptide, a cell culture wear and the like which are used for the method of the present invention. A reagent contained in the kit may be liquid or lyophilized.

A perforation method in the present invention can be used by appropriately selecting from an electroporation method, a microinjection method, a particle gun method and the like depending upon the purpose.

The present invention is illustrated by Examples below but is not limited to them.

45 Example 1

1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C-CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μM , respectively, which were sterilized using a 0.22 μm filter (Millex-GV, Millipore).

Each 1 ml/well of these solutions was added to a 24-well polystyrene culture dish (manufactured by Corning), respectively, to coat the dish at 4 °C overnight. These dishes were rinsed with a 500 μl /well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cells

Two culture dishes (diameter: 100 mm) of human epidermoid cancer cell A-431 which had been cultured in a Dul-

beco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 10 ml of PBS, a 3/10 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells were suspended again in 10 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 µg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 µF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of which were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added thereto, followed by culturing at 37 °C in the presence of 5% CO₂ gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT.

The results thereof are shown in Fig. 1. That is, Fig. 1 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 1, an amount of expressed CAT in the culture dish in the C274, H296 or C-CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 2

1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C-CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 µM, respectively, which were sterilized using a 0.22 µm filter (Millex-GV, Millipore). 1 ml/well of these solutions were added to a 24-well polystyrene culture dish (manufactured by Corning) to coat the dish at 4 °C overnight, respectively. These dishes were rinsed with 500 µl/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cell

Two culture dishes (diameter: 100 mm) of African green monkey kidney cell COS-7 which had been cultured in a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 12 ml of PBS, a 5/6 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells

were suspended in 6 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 µg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 µF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of the cells were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added, followed by culturing at 37 °C in the presence of 5% CO₂ gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT. The results thereof are shown in Fig. 2. That is, Fig. 2 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 2, an amount of expressed CAT in the culture dish in the above C274, H296 or C-CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 3

Preparation of kit

A kit for production of gene-transferred cells was made from C274, H296, C-CS1, PBS and a culturing dish as shown in Table 2 below. Reagents A, B and C were prepared so that the above polypeptides were adjusted with PBS to indicated concentrations shown in the Table. Other components were used which are described in Example 1. In addition, all of reagents A, B and C and a diluent for reagents were aseptically prepared by pre-filtering with a 0.22 µm sterile filter.

Table 2

Kit for production of transfected cell	
Reagent A . . . 100 µM C274	150 µl
Reagent B . . . 100 µM H296	150 µl
Reagent C . . . 100 µM C-CS1	150 µl
Diluent for reagents . . . PBS	45 ml
24-well polystyrene culture dish	3

As described above, the present invention can overcome the problems of the previous methods for gene transfer into cells and provide a method, for production of transfected cells, having improved efficacy of gene transfer into target cells. The present invention can also provide a kit, for production of transfected cells, which are used for the method.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into human epidermoid cancer cell A-431.

Fig. 2 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into African green monkey kidney cell COS-7.

Sequence Listing

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

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(B) STREET: 609, Takenaka-cho, Fushimi-ku

10 (C) CITY: Kyoto-shi, Kyoto

(E) COUNTRY: Japan

(F) ZIP: 612

(ii) TITLE OF INVENTION: Method for production of transfected cells

15 (iii) NUMBER OF SEQUENCES: 21

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb

20 (B) COMPUTER: IBM PS/2 Model 50Z or 55SX

(C) OPERATING SYSTEM: MS-DOS (Version 5.0)

(D) SOFTWARE: Microsoft Word

(v) CURRENT APPLICATION DATA:

25 (A) APPLICATION NUMBER: EP 95 93 8599.8

(B) FILING DATE:

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/JP95/02425

30 (B) FILING DATE: 29. November 1995

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 4

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

40 Arg Gly Asp Ser
1

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH: 25

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His
5 10 15
Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
55 20 25

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 274

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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10      Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
        1           5           10           15
      Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
        20           25           30
      Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
        35           40           45
15      Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
        50           55           60
      Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
        65           70           75
      His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
        80           85           90
20      Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
        95          100          105
      Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
       110          115          120
      Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
       125          130          135
25      Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
       140          145          150
      Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
       155          160          165
30      Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
       170          175          180
      Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
       185          190          195
      Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
       200          205          210
35      Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
       215          220          225
      Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
       230          235          240
      Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
       245          250          255
40      Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
       260          265          270
      Thr Glu Ile Asp

```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 296

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```

55      Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro
        5           10           15

```

	Thr	Ser	Leu	Ser	Ala	Gln	Trp	Thr	Pro	Pro	Asn	Val	Gln	Leu	Thr
					20					25					30
	Gly	Tyr	Arg	Val	Arg	Val	Thr	Pro	Lys	Glu	Lys	Thr	Gly	Pro	Met
5					35					40					45
	Lys	Glu	Ile	Asn	Leu	Ala	Pro	Asp	Ser	Ser	Ser	Val	Val	Val	Ser
					50					55					60
	Gly	Leu	Met	Val	Ala	Thr	Lys	Tyr	Glu	Val	Ser	Val	Tyr	Ala	Leu
					65					70					75
	Lys	Asp	Thr	Leu	Thr	Ser	Arg	Pro	Ala	Gln	Gly	Val	Val	Thr	Thr
10					80					85					90
	Leu	Glu	Asn	Val	Ser	Pro	Pro	Arg	Arg	Ala	Arg	Val	Thr	Asp	Ala
					95					100					105
	Thr	Glu	Thr	Thr	Ile	Thr	Ile	Ser	Trp	Arg	Thr	Lys	Thr	Glu	Thr
					110					115					120
15	Ile	Thr	Gly	Phe	Gln	Val	Asp	Ala	Val	Pro	Ala	Asn	Gly	Gln	Thr
					125					130					135
	Pro	Ile	Gln	Arg	Thr	Ile	Lys	Pro	Asp	Val	Arg	Ser	Tyr	Thr	Ile
					140					145					150
	Thr	Gly	Leu	Gln	Pro	Gly	Thr	Asp	Tyr	Lys	Ile	Tyr	Leu	Tyr	Thr
					155					160					165
20	Leu	Asn	Asp	Asn	Ala	Arg	Ser	Ser	Pro	Val	Val	Ile	Asp	Ala	Ser
					170					175					180
	Thr	Ala	Ile	Asp	Ala	Pro	Ser	Asn	Leu	Arg	Phe	Leu	Ala	Thr	Thr
					185					190					195
	Pro	Asn	Ser	Leu	Leu	Val	Ser	Trp	Gln	Pro	Pro	Arg	Ala	Arg	Ile
					200					205					210
25	Thr	Gly	Tyr	Ile	Ile	Lys	Tyr	Glu	Lys	Pro	Gly	Ser	Pro	Pro	Arg
					215					220					225
	Glu	Val	Val	Pro	Arg	Pro	Arg	Pro	Gly	Val	Thr	Glu	Ala	Thr	Ile
					230					235					240
	Thr	Gly	Leu	Glu	Pro	Gly	Thr	Glu	Tyr	Thr	Ile	Tyr	Val	Ile	Ala
					245					250					255
30	Leu	Lys	Asn	Asn	Gln	Lys	Ser	Glu	Pro	Leu	Ile	Gly	Arg	Lys	Lys
					260					265					270
	Thr	Asp	Glu	Leu	Pro	Gln	Leu	Val	Thr	Leu	Pro	His	Pro	Asn	Leu
					275					280					285
35	His	Gly	Pro	Glu	Ile	Leu	Asp	Val	Pro	Ser	Thr				
					290					295					

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 302

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

45	Pro	Thr	Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	Arg
	1				5					10					15
	Val	Thr	Trp	Ala	Pro	Pro	Pro	Ser	Ile	Asp	Leu	Thr	Asn	Phe	Leu
					20					25					30
	Val	Arg	Tyr	Ser	Pro	Val	Lys	Asn	Glu	Glu	Asp	Val	Ala	Glu	Leu
					35					40					45
50	Ser	Ile	Ser	Pro	Ser	Asp	Asn	Ala	Val	Val	Leu	Thr	Asn	Leu	Leu
					50					55					60
	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Val	Ser	Ser	Val	Tyr	Glu	Gln
					65					70					75

His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
 80 85 90
 Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
 95 100 105
 5 Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
 110 115 120
 Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
 125 130 135
 Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
 140 145 150
 10 Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
 155 160 165
 Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
 170 175 180
 Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
 185 190 195
 15 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
 200 205 210
 Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 215 220 225
 20 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
 230 235 240
 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
 245 250 255
 Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
 260 265 270
 25 Thr Glu Ile Asp Lys Pro Ser Asp Glu Leu Pro Gln Leu Val Thr
 275 280 285
 Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro
 290 295 300
 Ser Thr

30 (2) INFORMATION FOR SEQ ID NO: 6:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 35 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Ile Gly Ser Arg
 1 5

40 (2) INFORMATION FOR SEQ ID NO: 7:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 283
 (B) TYPE: amino acid
 45 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

50 Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro
 1 5 10 15
 Asp Thr Met Arg Val Thr Trp Ala Pro Pro Ser Ile Asp Leu
 20 25 30
 Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp

55

		35		40		45
	Val	Ala	Glu	Leu	Ser	Ile
		50		55		60
5	Thr	Asn	Leu	Leu	Pro	Gly
		65		70		75
	Val	Tyr	Glu	Gln	His	Glu
		80		85		90
	Thr	Gly	Leu	Asp	Ser	Pro
		95		100		105
10	Ala	Asn	Ser	Phe	Thr	Val
		110		115		120
	Thr	Gly	Tyr	Arg	Ile	Arg
		125		130		135
	Pro	Arg	Glu	Asp	Arg	Val
		140		145		150
15	Thr	Asn	Leu	Thr	Pro	Gly
		155		160		165
	Leu	Asn	Gly	Arg	Glu	Ser
		170		175		180
	Thr	Val	Ser	Asp	Val	Pro
		185		190		195
20	Pro	Thr	Ser	Leu	Leu	Ile
		200		205		210
	Arg	Tyr	Tyr	Arg	Ile	Thr
		215		220		225
	Val	Gln	Glu	Phe	Thr	Val
		230		235		240
25	Ser	Gly	Leu	Lys	Pro	Gly
		245		250		255
	Val	Thr	Gly	Arg	Gly	Asp
		260		265		270
30	Ile	Asn	Tyr	Arg	Thr	Glu
		275		280		

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 279

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

40	Pro	Thr	Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	Arg
	1				5					10				15	
	Val	Thr	Trp	Ala	Pro	Pro	Pro	Ser	Ile	Asp	Leu	Thr	Asn	Phe	Leu
				20						25				30	
	Val	Arg	Tyr	Ser	Pro	Val	Lys	Asn	Glu	Glu	Asp	Val	Ala	Glu	Leu
				35						40				45	
45	Ser	Ile	Ser	Pro	Ser	Asp	Asn	Ala	Val	Val	Leu	Thr	Asn	Leu	Leu
				50						55				60	
	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Val	Ser	Ser	Val	Tyr	Glu	Gln
				65						70				75	
	His	Glu	Ser	Thr	Pro	Leu	Arg	Gly	Arg	Gln	Lys	Thr	Gly	Leu	Asp
				80						85				90	
50	Ser	Pro	Thr	Gly	Ile	Asp	Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe
				95						100				105	
	Thr	Val	His	Trp	Ile	Ala	Pro	Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg

55

		110		115		120
	Ile Arg His His	Pro Glu His Phe Ser	Gly Arg Pro Arg Glu Asp			
		125		130		135
5	Arg Val Pro His	Ser Arg Asn Ser Ile Thr	Leu Thr Asn Leu Thr			
		140		145		150
	Pro Gly Thr Glu Tyr	Val Val Ser Ile Val	Ala Leu Asn Gly Arg			
		155		160		165
	Glu Glu Ser Pro	Leu Leu Ile Gly Gln	Gln Ser Thr Val Ser Asp			
		170		175		180
10	Val Pro Arg Asp	Leu Glu Val Val Ala	Ala Thr Pro Thr Ser Leu			
		185		190		195
	Leu Ile Ser Trp	Asp Ala Pro Ala Val	Thr Val Arg Tyr Tyr Arg			
		200		205		210
	Ile Thr Tyr Gly	Glu Thr Gly Gly Asn	Ser Pro Val Gln Glu Phe			
		215		220		225
15	Thr Val Pro Gly	Ser Lys Ser Thr Ala	Thr Ile Ser Gly Leu Lys			
		230		235		240
	Pro Gly Val Asp	Tyr Thr Ile Thr Val	Tyr Ala Val Thr Gly Arg			
		245		250		255
	Gly Asp Ser Pro	Ala Ser Ser Lys Pro	Ile Ser Ile Asn Tyr Arg			
		260		265		270
20	Thr Glu Ile Asp	Lys Pro Ser Gln Met				
		275				

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 474

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

	Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro	
	1 5 10	15
	Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu	
	20 25	30
35	Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp	
	35 40	45
	Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu	
	50 55	60
	Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser	
	65 70	75
40	Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys	
	80 85	90
	Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr	
	95 100	105
	Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile	
	110 115	120
45	Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg	
	125 130	135
	Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu	
	140 145	150
	Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala	
	155 160	165
50	Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser	
	170 175	180
	Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr	

		185		190		195
	Pro Thr Ser Leu	Leu Ile Ser Trp Asp	Ala Pro Ala Val Thr	Val		
		200		205		210
5	Arg Tyr Tyr Arg	Ile Thr Tyr Gly Glu	Thr Gly Gly Asn Ser	Pro		
		215		220		225
	Val Gln Glu Phe	Thr Val Pro Gly Ser	Lys Ser Thr Ala Thr	Ile		
		230		235		240
	Ser Gly Leu Lys	Pro Gly Val Asp Tyr	Thr Ile Thr Val Tyr	Ala		
		245		250		255
10	Val Thr Gly Arg	Gly Asp Ser Pro Ala	Ser Ser Lys Pro Ile	Ser		
		260		265		270
	Ile Asn Tyr Arg	Thr Glu Ile Asp Lys	Pro Ser Gln Asn Glu	Gly		
		275		280		285
	Leu Asn Gln Pro	Thr Asp Asp Ser Cys	Phe Asp Pro Tyr Thr	Val		
		290		295		300
15	Ser His Tyr Ala	Val Gly Asp Glu Trp	Glu Arg Met Ser Glu	Ser		
		305		310		315
	Gly Phe Lys Leu	Leu Cys Gln Cys Leu	Gly Phe Gly Ser Gly	His		
		320		325		330
	Phe Arg Cys Asp	Ser Ser Arg Trp Cys	His Asp Asn Gly Val	Asn		
		335		340		345
20	Tyr Lys Ile Gly	Glu Lys Trp Asp Arg	Gln Gly Glu Asn Gly	Gln		
		350		355		360
	Met Met Ser Cys	Thr Cys Leu Gly Asn	Gly Lys Gly Glu Phe	Lys		
		365		370		375
	Cys Asp Pro His	Glu Ala Thr Cys Tyr	Asp Asp Gly Lys Thr	Tyr		
		380		385		390
25	His Val Gly Glu	Gln Trp Gln Lys Glu	Tyr Leu Gly Ala Ile	Cys		
		395		400		405
	Ser Cys Thr Cys	Phe Gly Gly Gln Arg	Gly Trp Arg Cys Asp	Asn		
		410		415		420
	Cys Arg Arg Pro	Gly Gly Glu Pro Ser	Pro Glu Gly Thr Thr	Gly		
		425		430		435
30	Gln Ser Tyr Asn	Gln Tyr Ser Gln Arg	Tyr His Gln Arg Thr	Asn		
		440		445		450
	Thr Asn Val Asn	Cys Pro Ile Glu Cys	Phe Met Pro Leu Asp	Val		
		455		460		465
35	Gln Ala Asp Arg	Glu Asp Ser Arg Glu				
		470				

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 385

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

45	Ala Pro Ile Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr
	1 5 10 15
	Asn Leu His Leu Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val
	20 25 30
	Ser Trp Glu Arg Ser Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile
50	35 40 45
	Thr Thr Thr Pro Thr Asn Gly Gln Gln Gly Asn Ser Leu Glu Glu
	50 55 60
	Val Val His Ala Asp Gln Ser Ser Cys Thr Phe Asp Asn Leu Ser

55

	65	70	75
	Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr Thr Val Lys Asp Asp		
	80	85	90
5	Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile Pro Ala Val Pro		
	95	100	105
	Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met		
	110	115	120
	Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe		
	125	130	135
10	Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu		
	140	145	150
	Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu		
	155	160	165
	Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu		
	170	175	180
15	Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu		
	185	190	195
	Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser		
	200	205	210
	Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr		
	215	220	225
20	Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu		
	230	235	240
	Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu		
	245	250	255
	Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly		
25	260	265	270
	Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser		
	275	280	285
	Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser		
	290	295	300
30	Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr		
	305	310	315
	Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu		
	320	325	330
	Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu		
	335	340	345
35	Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly		
	350	355	360
	Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr		
	365	370	375
	Arg Thr Glu Ile Asp Lys Pro Ser Gln Met		
	380	385	

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(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 549

(B) TYPE: amino acid

45

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

50

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg	
1	5
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu	10
	15
	20
	25
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu	30

55

					35					40					45
	Ser	Ile	Ser	Pro	Ser	Asp	Asn	Ala	Val	Val	Leu	Thr	Asn	Leu	Leu
					50					55					60
5	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Val	Ser	Ser	Val	Tyr	Glu	Gln
					65					70					75
	His	Glu	Ser	Thr	Pro	Leu	Arg	Gly	Arg	Gln	Lys	Thr	Gly	Leu	Asp
					80					85					90
	Ser	Pro	Thr	Gly	Ile	Asp	Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe
					95					100					105
10	Thr	Val	His	Trp	Ile	Ala	Pro	Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg
					110					115					120
	Ile	Arg	His	His	Pro	Glu	His	Phe	Ser	Gly	Arg	Pro	Arg	Glu	Asp
					125					130					135
	Arg	Val	Pro	His	Ser	Arg	Asn	Ser	Ile	Thr	Leu	Thr	Asn	Leu	Thr
					140					145					150
15	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Ile	Val	Ala	Leu	Asn	Gly	Arg
					155					160					165
	Glu	Glu	Ser	Pro	Leu	Leu	Ile	Gly	Gln	Gln	Ser	Thr	Val	Ser	Asp
					170					175					180
	Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	Thr	Pro	Thr	Ser	Leu
					185					190					195
20	Leu	Ile	Ser	Trp	Asp	Ala	Pro	Ala	Val	Thr	Val	Arg	Tyr	Tyr	Arg
					200					205					210
	Ile	Thr	Tyr	Gly	Glu	Thr	Gly	Gly	Asn	Ser	Pro	Val	Gln	Glu	Phe
					215					220					225
25	Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	Leu	Lys
					230					235					240
	Pro	Gly	Val	Asp	Tyr	Thr	Ile	Thr	Val	Tyr	Ala	Val	Thr	Gly	Arg
					245					250					255
	Gly	Asp	Ser	Pro	Ala	Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	Arg
					260					265					270
30	Thr	Glu	Ile	Asp	Lys	Pro	Ser	Met	Ala	Ile	Pro	Ala	Pro	Thr	Asp
					275					280					285
	Leu	Lys	Phe	Thr	Gln	Val	Thr	Pro	Thr	Ser	Leu	Ser	Ala	Gln	Trp
					290					295					300
	Thr	Pro	Pro	Asn	Val	Gln	Leu	Thr	Gly	Tyr	Arg	Val	Arg	Val	Thr
					305					310					315
35	Pro	Lys	Glu	Lys	Thr	Gly	Pro	Met	Lys	Glu	Ile	Asn	Leu	Ala	Pro
					320					325					330
	Asp	Ser	Ser	Ser	Val	Val	Val	Ser	Gly	Leu	Met	Val	Ala	Thr	Lys
					335					340					345
	Tyr	Glu	Val	Ser	Val	Tyr	Ala	Leu	Lys	Asp	Thr	Leu	Thr	Ser	Arg
					350					355					360
40	Pro	Ala	Gln	Gly	Val	Val	Thr	Thr	Leu	Glu	Asn	Val	Ser	Pro	Pro
					365					370					375
	Arg	Arg	Ala	Arg	Val	Thr	Asp	Ala	Thr	Glu	Thr	Thr	Ile	Thr	Ile
					380					385					390
	Ser	Trp	Arg	Thr	Lys	Thr	Glu	Thr	Ile	Thr	Gly	Phe	Gln	Val	Asp
					395					400					405
45	Ala	Val	Pro	Ala	Asn	Gly	Gln	Thr	Pro	Ile	Gln	Arg	Thr	Ile	Lys
					410					415					420
	Pro	Asp	Val	Arg	Ser	Tyr	Thr	Ile	Thr	Gly	Leu	Gln	Pro	Gly	Thr
					425					430					435
	Asp	Tyr	Lys	Ile	Tyr	Leu	Tyr	Thr	Leu	Asn	Asp	Asn	Ala	Arg	Ser
					440					445					450
50	Ser	Pro	Val	Val	Ile	Asp	Ala	Ser	Thr	Ala	Ile	Asp	Ala	Pro	Ser
					455					460					465
	Asn	Leu	Arg	Phe	Leu	Ala	Thr	Thr	Pro	Asn	Ser	Leu	Leu	Val	Ser

55

		470		475		480
	Trp	Gln	Pro	Pro	Arg	Ala
				Arg	Ile	Thr
				Gly	Tyr	Ile
				Ile	Ile	Lys
				Tyr		
5	Glu	Lys	Pro	Gly	Ser	Pro
				Pro	Arg	Glu
				Val	Val	Pro
				Arg	Pro	Arg
				Pro	Arg	Pro
	Pro	Gly	Val	Thr	Glu	Ala
				Thr	Ile	Thr
				Gly	Leu	Glu
				Pro	Gly	Thr
				Thr		
	Glu	Tyr	Thr	Ile	Tyr	Val
				Ile	Ala	Leu
				Lys	Asn	Asn
				Gln	Lys	Ser
10	Glu	Pro	Leu	Ile	Gly	Arg
				Lys	Lys	Thr
				Thr		

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 422

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20	Pro	Thr	Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	Arg
	1				5					10					15
	Val	Thr	Trp	Ala	Pro	Pro	Pro	Ser	Ile	Asp	Leu	Thr	Asn	Phe	Leu
					20					25					30
	Val	Arg	Tyr	Ser	Pro	Val	Lys	Asn	Glu	Glu	Asp	Val	Ala	Glu	Leu
25					35					40					45
	Ser	Ile	Ser	Pro	Ser	Asp	Asn	Ala	Val	Val	Leu	Thr	Asn	Leu	Leu
					50					55					60
	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Val	Ser	Ser	Val	Tyr	Glu	Gln
					65					70					75
	His	Glu	Ser	Thr	Pro	Leu	Arg	Gly	Arg	Gln	Lys	Thr	Gly	Leu	Asp
30					80					85					90
	Ser	Pro	Thr	Gly	Ile	Asp	Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe
					95					100					105
	Thr	Val	His	Trp	Ile	Ala	Pro	Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg
					110					115					120
35	Ile	Arg	His	His	Pro	Glu	His	Phe	Ser	Gly	Arg	Pro	Arg	Glu	Asp
					125					130					135
	Arg	Val	Pro	His	Ser	Arg	Asn	Ser	Ile	Thr	Leu	Thr	Asn	Leu	Thr
					140					145					150
	Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Ile	Val	Ala	Leu	Asn	Gly	Arg
					155					160					165
40	Glu	Glu	Ser	Pro	Leu	Leu	Ile	Gly	Gln	Gln	Ser	Thr	Val	Ser	Asp
					170					175					180
	Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	Thr	Pro	Thr	Ser	Leu
					185					190					195
	Leu	Ile	Ser	Trp	Asp	Ala	Pro	Ala	Val	Thr	Val	Arg	Tyr	Tyr	Arg
					200					205					210
45	Ile	Thr	Tyr	Gly	Glu	Thr	Gly	Gly	Asn	Ser	Pro	Val	Gln	Glu	Phe
					215					220					225
	Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	Leu	Lys
					230					235					240
	Pro	Gly	Val	Asp	Tyr	Thr	Ile	Thr	Val	Tyr	Ala	Val	Thr	Gly	Arg
					245					250					255
50	Gly	Asp	Ser	Pro	Ala	Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	Arg
					260					265					270
	Thr	Glu	Ile	Asp	Lys	Pro	Ser	Met	Ala	Asn	Glu	Gly	Leu	Asn	Gln

275 280 285
 Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val Ser His Tyr
 290 295 300
 5 Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly Phe Lys
 305 310 315
 Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His Phe Arg Cys
 320 325 330
 Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr Lys Ile
 335 340 345
 10 Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met Ser
 350 355 360
 Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro
 365 370 375
 His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly
 380 385 390
 15 Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr
 395 400 405
 Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg
 410 415 420
 Pro Gly

20

(2) INFORMATION FOR SEQ ID NO: 13:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 332
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1 5 10 15
 30 Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
 20 25 30
 Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
 35 35 40 45
 Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
 50 55 60
 35 Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
 65 70 75
 His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
 80 85 90
 Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
 95 100 105
 40 Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
 110 115 120
 Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
 125 130 135
 45 Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
 140 145 150
 Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
 155 160 165
 Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
 170 175 180
 50 Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
 185 190 195
 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
 200 205 210

55

Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 215 220 225
 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
 230 235 240
 5 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
 245 250 255
 Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
 260 265 270
 10 Thr Glu Ile Asp Lys Pro Ser Met Ala Asn Ser Asp Ser Glu Cys
 275 280 285
 Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met
 290 295 300
 Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly
 305 310 315
 15 Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu
 320 325 330
 Leu Arg

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 341
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1 5 10 15
 Val Thr Trp Ala Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
 20 25 30
 30 Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
 35 40 45
 Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
 50 55 60
 Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
 65 70 75
 35 His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
 80 85 90
 Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
 95 100 105
 Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
 110 115 120
 40 Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
 125 130 135
 Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
 140 145 150
 Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
 155 160 165
 45 Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
 170 175 180
 Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
 185 190 195
 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
 200 205 210
 50 Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 215 220 225
 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys

230 235 240
 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
 245 250 255
 5 Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
 260 265 270
 Thr Glu Ile Asp Lys Pro Ser Met Gly Ile Tyr Ile Ser Gly Met
 275 280 285
 Ala Pro Arg Pro Ser Leu Thr Lys Lys Gln Arg Phe Arg His Arg
 290 295 300
 10 Asn Arg Lys Gly Tyr Arg Ser Gln Arg Gly His Ser Arg Gly Arg
 305 310 315
 Asn Gln Asn Ser Arg Arg Pro Ser Arg Ala Met Trp Leu Ser Leu
 320 325 330
 Phe Ser Ser Lys Asn Ser Ser Ser Val Pro Ala
 335 340

15

(2) INFORMATION FOR SEQ ID NO: 15:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 446
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

20

25 Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1 5 10 15
 Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
 20 25 30
 Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
 35 40 45
 30 Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
 50 55 60
 Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
 65 70 75
 His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
 80 85 90
 35 Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
 95 100 105
 Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
 110 115 120
 Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
 125 130 135
 40 Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
 140 145 150
 Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
 155 160 165
 Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
 170 175 180
 45 Val Pro Arg Asp Leu Glu Val Val Ala Thr Pro Thr Ser Leu
 185 190 195
 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
 200 205 210
 Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 215 220 225
 50 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
 230 235 240
 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg

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		245		250		255
	Gly Asp Ser Pro	Ala Ser Ser Lys Pro	Ile Ser Ile Asn Tyr Arg			
		260		265		270
5	Thr Glu Ile Asp	Lys Pro Ser Met Val	Pro Gly Phe Lys Gly Asp			
		275		280		285
	Met Gly Leu Lys	Gly Asp Arg Gly Glu	Val Gly Gln Ile Gly Pro			
		290		295		300
	Arg Gly Xxx Asp	Gly Pro Glu Gly Pro	Lys Gly Arg Ala Gly Pro			
		305		310		315
10	Thr Gly Asp Pro	Gly Pro Ser Gly Gln	Ala Gly Glu Lys Gly Lys			
		320		325		330
	Leu Gly Val Pro	Gly Leu Pro Gly Tyr	Pro Gly Arg Gln Gly Pro			
		335		340		345
	Lys Gly Ser Thr	Gly Phe Pro Gly Phe	Pro Gly Ala Asn Gly Glu			
		350		355		360
15	Lys Gly Ala Arg	Gly Val Ala Gly Lys	Pro Gly Pro Arg Gly Gln			
		365		370		375
	Arg Gly Pro Thr	Gly Pro Arg Gly Ser	Arg Gly Ala Arg Gly Pro			
		380		385		390
	Thr Gly Lys Pro	Gly Pro Lys Gly Thr	Ser Gly Gly Asp Gly Pro			
		395		400		405
20	Pro Gly Pro Pro	Gly Glu Arg Gly Pro	Gln Gly Pro Gln Gly Pro			
		410		415		420
	Val Gly Phe Pro	Gly Pro Lys Gly Pro	Pro Gly Pro Pro Gly Arg			
		425		430		435
	Met Gly Cys Pro	Gly His Pro Gly Gln	Arg Gly			
25		440		445		

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 457

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

35	Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg	
	1 5 10	15
	Val Thr Trp Ala Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu	
	20 25	30
	Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu	
	35 40	45
40	Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu	
	50 55	60
	Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln	
	65 70	75
	His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp	
	80 85	90
45	Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe	
	95 100	105
	Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg	
	110 115	120
	Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp	
	125 130	135
50	Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr	
	140 145	150
	Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg	

155 160 165
 Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
 170 175 180
 Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
 185 190 195
 Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
 200 205 210
 Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
 215 220 225
 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
 230 235 240
 Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
 245 250 255
 Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
 260 265 270
 Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg
 275 280 285
 Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp
 290 295 300
 Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val
 305 310 315
 Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
 320 325 330
 Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr
 335 340 345
 Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro
 350 355 360
 Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
 365 370 375
 Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln
 380 385 390
 Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys
 395 400 405
 Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly
 410 415 420
 Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr
 425 430 435
 Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro
 440 445 450
 Leu Ile Gly Arg Lys Lys Thr
 455

- (2) INFORMATION FOR SEQ ID NO: 17:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 368
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1 5 10 15
 Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
 20 25 30
 Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
 35 40 45
 Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu

		50		55		60
	Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln					
		65		70		75
5	His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp					
		80		85		90
	Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe					
		95		100		105
	Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg					
		110		115		120
10	Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp					
		125		130		135
	Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr					
		140		145		150
	Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg					
		155		160		165
15	Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp					
		170		175		180
	Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu					
		185		190		195
	Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg					
		200		205		210
20	Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe					
		215		220		225
	Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys					
		230		235		240
	Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg					
		245		250		255
25	Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg					
		260		265		270
	Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Asp Ala Pro Ser Asn					
		275		280		285
30	Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp					
		290		295		300
	Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu					
		305		310		315
	Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro					
		320		325		330
35	Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu					
		335		340		345
	Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu					
		350		355		360
	Pro Leu Ile Gly Arg Lys Lys Thr					
		365				

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(2) INFORMATION FOR SEQ ID NO: 18:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 367
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

45

50

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg			
1	5	10	15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu			
	20	25	30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu			

55

[illegible]

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 464
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
1 5 10 15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

		20		25		30
	Val Arg Tyr Ser Pro	Val Lys Asn Glu Glu Asp Val Ala Glu Leu				
		35		40		45
5	Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu					
		50		55		60
	Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln					
		65		70		75
	His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp					
		80		85		90
10	Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe					
		95		100		105
	Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg					
		110		115		120
	Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp					
		125		130		135
15	Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr					
		140		145		150
	Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg					
		155		160		165
	Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp					
		170		175		180
20	Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu					
		185		190		195
	Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg					
		200		205		210
	Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe					
		215		220		225
25	Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys					
		230		235		240
	Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg					
		245		250		255
	Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg					
		260		265		270
30	Thr Glu Ile Asp Lys Pro Ser Met Gly Ile Arg Gly Leu Lys Gly					
		275		280		285
	Thr Lys Gly Glu Lys Gly Glu Asp Gly Phe Pro Gly Phe Lys Gly					
		290		295		300
35	Asp Met Gly Ile Lys Gly Asp Arg Gly Glu Ile Gly Pro Pro Gly					
		305		310		315
	Pro Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Gly					
		320		325		330
	Pro Asn Gly Asp Pro Gly Pro Leu Gly Pro Pro Gly Glu Lys Gly					
		335		340		345
40	Lys Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly					
		350		355		360
	Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly					
		365		370		375
	Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gly					
		380		385		390
45	Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly					
		395		400		405
	Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gly					
		410		415		420
	Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gly					
		425		430		435
50	Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly					
		440		445		450
	Lys Asp Gly Leu Pro Gly His Pro Gly Gln Arg Gly Glu Thr					

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455

460

(2) INFORMATION FOR SEQ ID NO: 20:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 432

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Pro	Thr	Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	Arg	1	5	10	15
Val	Thr	Trp	Ala	Pro	Pro	Pro	Ser	Ile	Asp	Leu	Thr	Asn	Phe	Leu	20	25	30	35
Val	Arg	Tyr	Ser	Pro	Val	Lys	Asn	Glu	Glu	Asp	Val	Ala	Glu	Leu	40	45	50	55
Ser	Ile	Ser	Pro	Ser	Asp	Asn	Ala	Val	Val	Leu	Thr	Asn	Leu	Leu	60	65	70	75
Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Val	Ser	Ser	Val	Tyr	Glu	Gln	80	85	90	95
His	Glu	Ser	Thr	Pro	Leu	Arg	Gly	Arg	Gln	Lys	Thr	Gly	Leu	Asp	100	105	110	115
Ser	Pro	Thr	Gly	Ile	Asp	Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe	120	125	130	135
Thr	Val	His	Trp	Ile	Ala	Pro	Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg	140	145	150	155
Ile	Arg	His	His	Pro	Glu	His	Phe	Ser	Gly	Arg	Pro	Arg	Glu	Asp	160	165	170	175
Arg	Val	Pro	His	Ser	Arg	Asn	Ser	Ile	Thr	Leu	Thr	Asn	Leu	Thr	180	185	190	195
Pro	Gly	Thr	Glu	Tyr	Val	Val	Ser	Ile	Val	Ala	Leu	Asn	Gly	Arg	200	205	210	215
Glu	Glu	Ser	Pro	Leu	Ile	Gly	Gln	Gln	Ser	Thr	Val	Ser	Asp		220	225	230	235
Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	Thr	Pro	Thr	Ser	Leu	240	245	250	255
Leu	Ile	Ser	Trp	Asp	Ala	Pro	Ala	Val	Thr	Val	Arg	Tyr	Tyr	Arg	260	265	270	275
Ile	Thr	Tyr	Gly	Glu	Thr	Gly	Gly	Asn	Ser	Pro	Val	Gln	Glu	Phe	280	285	290	295
Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	Leu	Lys	300	305	310	315
Pro	Gly	Val	Asp	Tyr	Thr	Ile	Thr	Val	Tyr	Ala	Val	Thr	Gly	Arg	320	325	330	335
Gly	Asp	Ser	Pro	Ala	Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	Arg	340	345	350	355
Thr	Glu	Ile	Asp	Lys	Pro	Ser	Met	Ala	Ala	Gly	Ser	Ile	Thr	Thr	360	365	370	375
Leu	Pro	Ala	Leu	Pro	Glu	Asp	Gly	Gly	Ser	Gly	Ala	Phe	Pro	Pro	380	385	390	395
Gly	His	Phe	Lys	Asp	Pro	Lys	Arg	Leu	Tyr	Cys	Lys	Asn	Gly	Gly	400	405	410	415
Phe	Phe	Leu	Arg	Ile	His	Pro	Asp	Gly	Arg	Val	Asp	Gly	Val	Arg	420	425	430	435
Glu	Lys	Ser	Asp	Pro	His	Ile	Lys	Leu	Gln	Leu	Gln	Ala	Glu	Glu	440	445	450	455
Arg	Gly	Val	Val	Ser	Ile	Lys	Gly	Val	Cys	Ala	Asn	Arg	Tyr	Leu	460	465	470	475

		350		355		360
	Ala Met Lys Glu Asp Gly Arg Leu Leu		Ala Ser Lys Cys Val Thr			
		365		370		375
5	Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn					
		380		385		390
	Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys					
		395		400		405
	Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln					
		410		415		420
10	Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser					
		425		430		

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 574

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

20	Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg	
	1 5 10	15
	Val Thr Trp Ala Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu	
	20 25	30
	Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu	
25	35 40	45
	Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu	
	50 55	60
	Pro Gly Thr Glu Tyr Val Val Ser Val Ser Val Tyr Glu Gln	
	65 70	75
	His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp	
30	80 85	90
	Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe	
	95 100	105
	Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg	
	110 115	120
35	Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp	
	125 130	135
	Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr	
	140 145	150
	Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg	
	155 160	165
40	Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp	
	170 175	180
	Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu	
	185 190	195
	Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg	
	200 205	210
45	Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe	
	215 220	225
	Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys	
	230 235	240
	Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg	
50	245 250	255
	Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg	
	260 265	270
	Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Pro Ala Pro Thr Asp	

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					275					280				285					
					Leu	Lys	Phe	Thr	Gln	Val	Thr	Pro	Thr	Ser	Leu	Ser	Ala	Gln	Trp
					290									295					300
5					Thr	Pro	Pro	Asn	Val	Gln	Leu	Thr	Gly	Tyr	Arg	Val	Arg	Val	Thr
					305									310					315
					Pro	Lys	Glu	Lys	Thr	Gly	Pro	Met	Lys	Glu	Ile	Asn	Leu	Ala	Pro
					320									325					330
					Asp	Ser	Ser	Ser	Val	Val	Val	Ser	Gly	Leu	Met	Val	Ala	Thr	Lys
10					335									340					345
					Tyr	Glu	Val	Ser	Val	Tyr	Ala	Leu	Lys	Asp	Thr	Leu	Thr	Ser	Arg
					350									355					360
					Pro	Ala	Gln	Gly	Val	Val	Thr	Thr	Leu	Glu	Asn	Val	Ser	Pro	Pro
					365									370					375
15					Arg	Arg	Ala	Arg	Val	Thr	Asp	Ala	Thr	Glu	Thr	Thr	Ile	Thr	Ile
					380									385					390
					Ser	Trp	Arg	Thr	Lys	Thr	Glu	Thr	Ile	Thr	Gly	Phe	Gln	Val	Asp
					395									400					405
					Ala	Val	Pro	Ala	Asn	Gly	Gln	Thr	Pro	Ile	Gln	Arg	Thr	Ile	Lys
20					410									415					420
					Pro	Asp	Val	Arg	Ser	Tyr	Thr	Ile	Thr	Gly	Leu	Gln	Pro	Gly	Thr
					425									430					435
					Asp	Tyr	Lys	Ile	Tyr	Leu	Tyr	Thr	Leu	Asn	Asp	Asn	Ala	Arg	Ser
					440									445					450
25					Ser	Pro	Val	Val	Ile	Asp	Ala	Ser	Thr	Ala	Ile	Asp	Ala	Pro	Ser
					455									460					465
					Asn	Leu	Arg	Phe	Leu	Ala	Thr	Thr	Pro	Asn	Ser	Leu	Leu	Val	Ser
					470									475					480
					Trp	Gln	Pro	Pro	Arg	Ala	Arg	Ile	Thr	Gly	Tyr	Ile	Ile	Lys	Tyr
30					485									490					495
					Glu	Lys	Pro	Gly	Ser	Pro	Pro	Arg	Glu	Val	Val	Pro	Arg	Pro	Arg
					500									505					510
					Pro	Gly	Val	Thr	Glu	Ala	Thr	Ile	Thr	Gly	Leu	Glu	Pro	Gly	Thr
					515									520					525
35					Glu	Tyr	Thr	Ile	Tyr	Val	Ile	Ala	Leu	Lys	Asn	Asn	Gln	Lys	Ser
					530									535					540
					Glu	Pro	Leu	Ile	Gly	Arg	Lys	Lys	Thr	Asp	Glu	Leu	Pro	Gln	Leu
					545									550					555
					Val	Thr	Leu	Pro	His	Pro	Asn	Leu	His	Gly	Pro	Glu	Ile	Leu	Asp
40					560									565					570
					Val	Pro	Ser	Thr											

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Claims

1. In a method for production of transfected cells by transferring a foreign gene into target cells using a perforation method, said method for production of cells transfected with a foreign gene which comprises a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance.
2. The method for production of transfected cells according to claim 1, the culturing step is a step of culturing using a culture wear covered with a cell-adhering active substance.
3. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is a cell-adhering active polypeptide or a functional equivalent of said polypeptide.
4. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is

a cell-adhering and/or cell-spreading active polypeptide.

- 5 5. The method for production of transfected cells according to claim 3, wherein the cell-adhering and/or cell-spreading active polypeptide is a polypeptide containing the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.
6. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is selected from polypeptides represented by SEQ ID: Nos. 3, 4 and 5.
- 10 7. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is poly-N-p-vinylbenzyl-D-lactoneamide.
8. The method for production of transfected cells according to claim 1, wherein the target cells are selected from hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte and cancer cell.
- 15 9. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes).
- 20 10. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes) and the nucleic acid is incorporated into the vector.
- 25 11. The method for production of transfected cells according to claim 1, wherein the vector is a vector selected from retrovirus vector, adenovirus vector, vacciniavirus vector and herpesvirus vector.
12. The method for production of transfected cells according to claim 1, the perforation method is selected from an electroporation method, a microinjection method and a particle gun method.
- 30 13. Transfected cells produced by a method for production of transfected cells according to claim 1.
14. A kit for production of transfected cells with a foreign gene which is used in a method for production of transfected cells according to claim 1, said kit comprises containing a cell-adhering active substance.
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Fig. 1

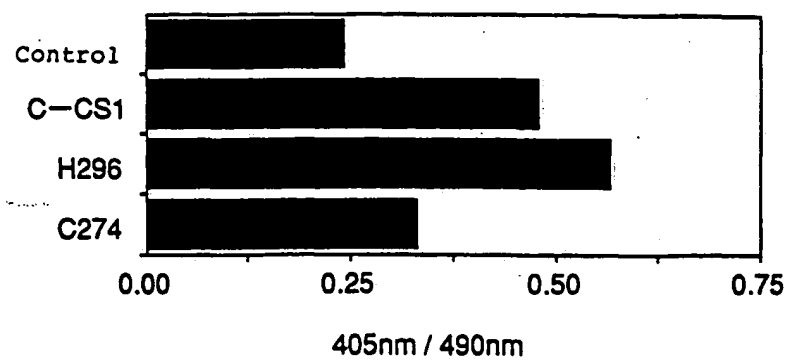
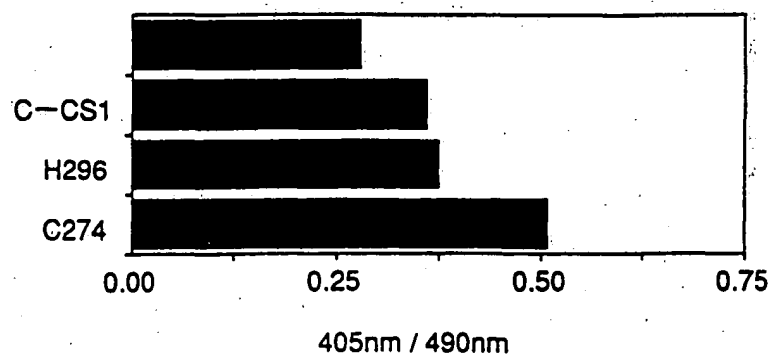


Fig. 2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/02425

A. CLASSIFICATION OF SUBJECT MATTERInt. Cl⁶ C12N15/87, C12N5/10, C07K14/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl⁶ C12N15/87, C12N5/10, C07K14/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, WPI/L, BIOSIS PREVIEWS
CAS ONLINE**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 4-063597, A (W.R. Grace & Co.), February 28, 1992 (28. 02. 92) & EP, 463508, A & CA, 2044307, A	1 - 14
A	JP, 6-090771, A (Shiseido Co., Ltd.), April 5, 1994 (05. 04. 94) (Family: none)	1 - 14

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"A" document member of the same patent family

Date of the actual completion of the international search

March 1, 1996 (01. 03. 96)

Date of mailing of the international search report

March 19, 1996 (19. 03. 96)

Name and mailing address of the ISA/

Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

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